Application No.: 09/911,039 Docket No.: POLYPROBE 3.0-017 CIP CONT CONT

IN THE SPECIFICATION

ENTER MARKED-UP VERSION OF AMENDED SPECIFICATION HERE.

[0001] This application is a continuation of U.S. Application Serial No. 09/482,803, filed January 13, 2000, abandoned, which is a continuation of U.S. Application No. 08/909,539, filed August 12, 1997, now U.S. Patent 6,046,038, which is a continuation-in-part of U.S. Application Serial No. 08/657,961, filed June 4, 1996, now U.S. Patent 6,072,043, which is incorporated herein by reference.

[0046] A "label" or "labeling moiety" is any compound employed as a means for detecting an oligonucleotide. The label or labeling moiety is attached to an oligonucleotide via ionic or covalent interactions, including hydrogen bonding, electrostatic interactions and intercalation. Examples of labels and labeling moieties include, but are not limited to fluorescent dye conjugates, biotin, digoxigenin, radionucleotides, antibodies, enzymes and receptors, such that detection of the labeled oligonucleotide is by fluorescence, conjugation to ~~streptaniden~~streptaviden and/or avidin, antigen-antibody and/or antibody-secondary antibody interactions, quantitation of radioactivity, catalytic and/or ligand-receptor interactions.

[0058] Labeling by ligation is accomplished by first synthesizing and purifying an optimally labeled nucleic acid (20 bases to 2 kilobases). The target sequence for labeling with the labeled oligonucleotide is nicked into small pieces, which average 30-70 bases, by chemical degradation or by treatment with nuclease such as DNAse I or a restriction enzyme. Approximately equal weights of labeled oligonucleotide (typically 50ng to 5µg in 50-100µl total reaction volume) and target sequence are reacted in ligation buffer as recommended by the ligase enzyme manufacturer. The relative success of the ligation step can be assessed by gel electrophoresis. The

ligated material can be directly used in hybridization assays or, if desired, purified by precipitation, size fractionation, gel electrophoresis, ~~antigen- specific~~ antigen-specific binding, or another method.

Labeling a Target Sequence by Randomer Extension

[0059] The basis of this labeling technique is the use of a short (6-12 base) random sequence at the 3′ end of the optimally labeled oligonucleotide. The initial labeling reaction of the template with label is modified such that the template molecule is designed to have a 5′ overhang (the extension region for incorporation of dye-NTP) as well as a 3′ overhang of 6-200 bases with the most 3′ sequence being a random sequence of typically ~~6--12~~-6-12 bases. The purified labeled oligonucleotide may be used directly in the primer extension reaction or preferably crosslinked with trimethylpsoralen prior to use in the target labeling reaction.